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I, DAVID DANIEL CLARKE , ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed are true copies of the Provisional specification and drawing(s) as filed on 30 November 1995 in connection with Application No. PN 6910 for a patent by DARETECH PTY. LTD. filed on 30 November 1995.

I further certify that the name of the applicant has been amended to DARATECH PTY. LTD., and PIG RESEARCH AND DEVELOPMENT CORPORATION pursuant to the provisions of Section 104 of the Patents Act 1990.

I further certify that the annexed documents are not, as yet, open to public inspection.

PRIORITY DOCUMENT

WITNESS my hand this Eighteenth day of December 1996

DAVID DANIEL CLARKE
ASSISTANT DIRECTOR PATENT SERVICES

Regulation 3.2

DARATECH PTY. LTD. and Pig Research and Development Corporation

AUSTRALIA
Patents Act 1990

PROVISIONAL SPECIFICATION
for the invention entitled:

"THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS - A"

The invention is described in the following statement:

THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS - A

The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in pigs caused or exacerbated by *Lawsonia intracellularis* or similar organism. The present invention also contemplates methods for the treatment and/or prophylaxis of such intestinal diseases and to diagnostic agents and procedures for detecting *Lawsonia intracellularis* or similar organism.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The meat industry in Australia and, indeed, in most countries of the world, is an important aspect of the overall livestock industry. However, the meat industry is subject to rapid economic downturn in response to disease conditions affecting the animals as well as human diseases putatively carried by the animals.

Pigs form a major component of the meat industry. However, pigs are sensitive to a wide spectrum of colonic diseases collectively referred to as porcine proliferative enteritis (PPE). This disease has previously been known as intestinal adentomatosis complex (1), porcine intestinal adenomatosis, necrotic enteritis (2), proliferative haemorrhagic enteropathy (3), regional ileitis (4), haemorrhagic bowel syndrome (5), porcine proliferative enteropathies and *Campylobacter* spp induced enteritis (6). There are two main forms of PPE: a non-haemorrhagic form represented by intestinal

adenomatosis which frequently causes growth retardation and mild diarrhoea; and a haemorrhagic form, which is often fatal, represented by proliferative haemorrhagic enteropathy (PHE) where the distal small intestine lumen becomes engorged with blood. PPE has been reported in a number of animal species including pigs (14), hamsters (7), ferrets (15), guinea pigs (16), rabbits (17) as well as avian species (18).

The causative organism of PPE is a *Campylobacter*-like organism referred to herein as *"Lawsonia intracellularis"* (26). The organism is also previously referred to as *Ileal symbiont intracellularis* (7). PPE-like diseases in pigs may also be caused by other pathogens such as various species of *Campylobacter* (8).

Lawsonia intracellularis is an intracellular, possibly obligate intracellular, bacterium. It can only be cultured *in vitro* with tissue culture cells (9, 26). Pigs suffering from PPE are characterised by multiple abnormal immature crypts and *L. intracellularis* is located in the cytoplasm of these cells.

PPE is a significant cost component associated with the pig industry, especially in terms of stock losses, medication costs, reduced growth rates of pigs and increased feed costs. PPE also contributes to downstream indirect costs in, for example, additional labour costs and environmental costs in dealing with antibiotic residue contamination and in control measures to prevent the organism being passed on or carried to other animals or humans.

Current control strategies for PPE rely on the use of antibiotics. However, such a strategy is considered to be short to medium term especially as governmental regulatory pressures tend to target animal husbandry practices which are only supported by prophylactic antibiotics. There is a need, therefore, to develop effective, safe and low cost alternatives to the use of antibiotics.

In work leading up to the present invention, the inventors sought to develop vaccines for the prophylaxis and treatment of PPE in pigs. The vaccines of the present invention provide an efficacious alternative to the use of antibiotics with a range of consequential

husbandry benefits.

Accordingly, one aspect of the present invention provides a vaccine composition for the prophylaxis and/or treatment of infection in a pig or similar animal by *L. intracellularis* or related microorganism, said vaccine composition comprising at least one immunogenic component from *L. intracellularis* or related microorganism and one or more carriers, diluents and/or adjuvants suitable for veterinary use.

The present invention is particularly useful and is exemplified hereinafter in relation to the protection and/or treatment of pigs from infection with *L. intracellularis*. However, this is done with the understanding that the present invention extends to the prophylaxis and treatment of animals related or similar to pigs from infection with *L. intracellularis* and/or related microorganisms. Reference hereinafter to pigs, therefore, includes animals related thereto.

Furthermore, the present invention extends to all isolates and sub-types of *L. intracellularis* as well as other species of the genus *Lawsonia* or other microorganisms related at the nucleotide, biochemical, structural, physiological and/or immunointeractive level. Reference hereinafter to *"Lawsonia intracellularis"* or its abbreviation *"L. intracellularis"* includes all microorganisms related to this microorganism. For example, a related organism may have a nucleotide sequence similarity at the chromosome or extrachromosomal level of at least about 60%, more preferably at least about 70% and even more preferably greater than at least about 80% with respect to all or part of a nucleotide sequence within the chromosome or extrachromosomal elements of *L. intracellularis*. For example, these percentage similarities may relate to the sequence set forth in SEQ ID NO: 7. This sequence is a portion of the *L. intracellularis* chromosome.

Accordingly, this aspect of the present invention is directed to a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis*, said vaccine composition comprising at least one immunogenic component from *L. intracellularis* and one or more carriers, diluents and/or adjuvants suitable for veterinary use.

The term "immunogenic component" refers to a component of *L. intracellularis* including a peptide, polypeptide or a protein encoded by DNA from or derived from *L. intracellularis* which is capable of inducing a protective immune response in a pig. A protective immune response may be at the humoural and/or cellular level and generally results in a substantial reduction in the symptoms of PPE in pigs. The vaccine compositions will comprise an effective amount of immunogenic component such as to permit induction of a protective immune response.

Accordingly, the present invention is directed to a vaccine composition for the prophylaxis and treatment of a pig by *L. intracellularis*, said vaccine composition comprising an amount of at least one immunogenic component from *L. intracellularis* effective to induce a protective immune response in said pig against *L. intracellularis*, said vaccine composition further comprising one or more carriers, adjuvants and/or diluents suitable for veterinary use.

The immunogenic component may be a naturally occurring peptide, polypeptide or protein, a carbohydrate, lipid or nucleic acid (e.g. DNA) or any combination thereof isolated from *L. intracellularis* or a cell culture thereof or a recombinant form of a peptide, polypeptide or protein encoded by DNA from or derived from *L. intracellularis* or is a derivative of said peptide, polypeptide or protein.

An isolated component of *L. intracellularis* is a component which has undergone at least one purification step or which has undergone at least partial concentration from a cell culture comprising *L. intracellularis* or from a lysed preparation of *L. intracellularis* cells. The purity of such a component from *L. intracellularis* which has the requisite immunogenic properties is preferably at least about 40%, more preferably at least about 50%, even more preferably at least about 60%, still more preferably at least about 70% and even more preferably at least about 80-90% or greater relative to other components in a preparation as determined by molecular weight, immunogenic activity or other suitable means.

A particularly useful form of the vaccine is a whole cell vaccine which comprises *L. intracellularis* in attenuated form or killed cells or various fractions thereof.

In one preferred embodiment, the vaccine comprises killed *L. intracellularis* cells prepared, for example, by heat, formalin or other chemical treatment, electric shock or pressure and such a vaccine is particularly useful in the practice of the present invention.

According to this aspect of the present invention there is provided a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis*, said vaccine composition comprising a killed preparation of *L. intracellularis* or an immunogenic fraction thereof and one or more carriers, diluents and/or adjuvants suitable for veterinary use.

In an alternative embodiment, a recombinant vaccine may be employed. The recombinant vaccine may comprise one or more recombinant peptides, polypeptides or proteins derived from *L. intracellularis* or is a recombinant molecule immunologically related to a peptide, polypeptide or protein derived from *L. intracellularis* or may be a fusion molecule having a first portion comprising a peptide, polypeptide or protein derived from *L. intracellularis* and a second heterologous peptide, polypeptide or protein which may be useful, for example, as a carrier molecule or an adjuvant or an immune stimulating molecule such as cytokine. A particularly useful recombinant protein from *L. intracellularis* comprises a peptide, polypeptide or protein derived from the cell surface or membrane of *L. intracellularis*, is an enzyme in a metabolic pathway within *L. intracellularis* or is a refolding and/or heatshock protein. In a preferred embodiment, the protein is a refolding/heatshock protein such as GroEL and GroES.

According to a preferred embodiment, the present invention relates to a vaccine composition for the prophylaxis and/or treatment of infection in a pig by *L. intracellularis*, said vaccine composition comprising at least one recombinant peptide, polypeptide or protein from *L. intracellularis* and wherein said recombinant peptide, polypeptide or protein is capable of inducing a protective immune response against *L. intracellularis* in pigs, the vaccine composition further comprising one or more carriers,

diluents and/or adjuvants suitable for veterinary use.

In a particularly preferred embodiment, the recombinant protein is GroEL having an amino acid sequence as set forth in SEQ ID NO:2 or is a protein having an amino acid sequence with at least about 40%, at least about 60%, or more preferably at least about 70% and even more preferably at least about 80-90% or greater similarity to all or part of the amino acid sequence set forth in SEQ ID NO:2.

In another embodiment, the recombinant molecule is GroES having an amino acid sequence as set forth in SEQ ID NO:4 or is a molecule having an amino acid sequence at least about 40%, at least about 60%, more preferably at least about 70% and even more preferably at least about 80-90% or greater similarity to all or part of the amino acid sequence set forth in SEQ ID NO:4.

The present invention further extends to a vaccine comprising a recombinant vaccine vector encoding a peptide, polypeptide or protein derived from *L. intracellularis* as described above. The vaccine vector may be of viral, yeast or bacterial origin and would be capable of expression of a genetic sequence encoding a peptide, polypeptide or protein from *L. intracellularis* in a manner effective to induce a protective immune response. For example, a non-pathogenic bacterium could be prepared containing a recombinant sequence capable of encoding a peptide, polypeptide or protein from *L. intracellularis*. The recombinant sequence would be in the form of an expression vector under the control of a constitutive or inducible promoter. The bacterium would then be permitted to colonise suitable locations in a pig's gut and would be permitted to grow and produce the recombinant peptide, polypeptide or protein in amount sufficient to induce a protective immune response against *L. intracellularis*.

In a further alternative embodiment, the vaccine may be a DNA vaccine comprising a DNA molecule encoding a peptide, polypeptide or protein from *L. intracellularis* and which is injected into muscular tissue or other suitable tissue in a pig under conditions sufficient to permit transient expression of said DNA to produce an amount of peptide, polypeptide or protein effective to induce a protective immune response.

The vaccines of the present invention may contain a single peptide, polypeptide or protein or a range of peptides, polypeptides or proteins covering different or similar epitopes. In addition, or alternatively, a single polypeptide may be provided with multiple epitopes. The latter type of vaccine is referred to as a polyvalent vaccine. A multiple epitope includes two or more repeating epitopes.

The formation of vaccines is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pennsylvania, USA.

The present invention, therefore, contemplates a pharmaceutical composition or vaccine composition comprising an immunity developing effective amount of one or more of:

(i) an immunogenic component from *L. intracellularis*;

(ii) a recombinant peptide, polypeptide or protein from *L. intracellularis* having immunogenic properties; and/or

(iii) whole cells or a component or fraction thereof from *L. intracellularis*.

The above components are referred to hereinafter as "active ingredients". The active ingredients of a vaccine composition as contemplated herein exhibit excellent therapeutic activity, for example, in the treatment and/or prophylaxis of PPE when administered in an amount which depends on the particular case. For example, for recombinant molecules, from about 0.5 μg to about 20 mg per kilogram of body weight per day may be administered. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. Booster administration may also be required.

The active ingredients may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or implanting (eg using slow release technology). Depending on the route of administration, the active ingredients which comprise, for example, peptides,

polypeptides or proteins may be required to be coated in a material to protect said ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredients.

The term "adjuvant" is used in its broadest sense and includes any immune stimulating compound such as interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether and Freund's complete and incomplete adjuvant.

The active compounds may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be fluid to the extent that easy syringability exists unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed. In any event, it must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying

absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

Carriers and diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents in vaccines is well known in the art. Except insofar as any conventional media or agent is incompatible with an active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

Still another aspect of the present invention is directed to antibodies to the peptides, polypeptides or proteins from *L. intracellularis* or recombinant forms thereof or non-proteinaceous molecules such as carbohydrates. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to *L. intracellularis* or may be specifically raised to specific molecules or whole cells or components or fractions thereof. The antibodies of the present invention are particularly useful for immunotherapy and vaccination and may also be used as a diagnostic tool for infection or for monitoring the progress of a vaccination or therapeutic regime.

For example, recombinant *L. intracellularis* peptides, polypeptides or proteins can be used to screen for naturally occurring antibodies to *L. intracellularis.* Alternatively, specific antibodies can be used to screen for *L. intracellularis.* Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA.

Hereinafter, an immunogenic component is considered to encompass an immunogenic component of *L intracellularis* and includes recombinant molecules, whole cells and cell extracts.

In accordance with this aspect of the present invention, the immunogenic components are particularly useful in screening for antibodies to *L. intracellularis* and, hence, provide a diagnostic protocol for detecting *L. intracellularis* infection. Alternatively, biological samples can be directly screened for *L. intracellularis* using antibodies raised to immunogenic components.

Accordingly, there is provided a method for the diagnosis of *L. intracellularis* infection in a pig comprising contacting a biological sample from said pig with an immunogenic component binding effective amount of an antibody for a time and under conditions sufficient for an immunogenic component-antibody complex to form, and then detecting said complex.

The presence of immunogenic components (or antibodies thereto) in a pig's blood, serum, or other bodily fluid, can be detected using a wide range of immunoassay techniques such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. This includes both single-site and two-site, or "sandwich", assays of the non-competitive types, as well as in the traditional competitive binding assays. Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention.

Briefly, in a typical forward assay, an immunogenic component-specific antibody is immobilised onto a solid substrate to form a first complex and the sample to be tested for immunogenic component brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-immunogenic component secondary complex, a second immunogenic component antibody, labelled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing sufficient time for the formation of a

tertiary complex. Any unreacted material is washed away, and the presence of bound labelled antibody is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparing with a control sample. The present invention contemplates a range of variations to the subject assay including an assay for *L. intracellularis* antibodies using, for example, recombinant peptides, polypeptides or proteins from this organism.

The solid substrate is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecule in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates, which yield a fluorescent product.

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody

adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining ternary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

The present invention is further described by the following non-limiting Figures and/or Examples.

In the Figures:

Figure 1 is a representation of the nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence of GroEL [SEQ ID NO:2].

Figure 2 is a representation of the nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence of GroES [SEQ ID NO:2].

Figure 3 is a photographic representation showing Western analysis of *L. intracellularis* antigens recognised by vaccinated pigs. Track 1 was probed with pig sera from a pig (395) that had been immunised three times with the formalin killed whole *L. intracellularis* vaccine. Track 2 to 5 were probed with sera obtained from pigs Y10, Y12, Y14 and Y16, respectively on day 0.

Figure 4 is a photographic representation of the small intestine obtained from pig Y1 on day 20.

Figure 5 is a photographic representation of the small intestine obtained from pig Y2 on day 20.

Figure 6 is a photographic representation of the small intestine obtained from pig Y4 on day 20.

Figure 7 is a graphical representation showing end titres of GroEL antibodies in the serum of pigs at day 2 □ and day 17 ■ of the vaccine trial.

Figure 8 is a graphical representation showing T-cell proliferation responses to GroEL and GST proteins by peripheral blood lymphocytes obtained from pigs on day 2 ■ rGroEL □ GST.

Figure 9 is a schematic representation of the plasmid pET-EL2 which contains the GroEL gene from *L. intracellularis*.

Figure 10 is a schematic representation of the plasmid pCIGH-EL2 which contains the GroEL gene from *L. intracellularis* driven by the CMU promoter.

The following single and three letter abbreviations are used for amino acid residues:

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

EXAMPLE 1

SOURCES OF PIG TISSUE

Infected Pig Intestines

Sections of grossly thickened ilea were taken from pigs naturally or experimentally affected by PPE. The presence of *L. intracellularis* bacteria in the ilea was confirmed using modified acid-fast Ziehl-Neelson staining, and/or immunofluorescent staining with specific monoclonal antibodies (10). An example of a suitable antibody is monoclonal antibody IG4 available from the University of Edinburgh, UK.

Normal Pig Intestines

Ilea from six pigs not infected with the disease were obtained from a quarantined piggery which has had no recorded outbreak of proliferative enteritis (Victorian Institute of Animal Science, Werribee, Australia).

EXAMPLE 2

ISOLATION OF *LAWSONIA INTRACELLULARIS* BACTERIA FROM THE INFECTED PIG ILIEUM

Lawsonia intracellularis bacteria were extracted directly from lesions of proliferative enteritis in pigs by filtration and further purified over a percoll (Pharmacia, Uppsala, Sweden) gradient. Infected ilea were collected from pigs and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 8g of infected mucosa were scraped from the intestinal wall. The mucosa was homogenised with 40 ml sterile phosphate buffered saline (PBS) on half speed for 10 s using a Sorvall omnimixer. This suspension was centrifuged at 2000 xg for 4 minutes. The supernatant was discarded and the cell pellet was resuspended in 40 ml PBS and recentrifuged. This washing step was repeated twice. The cell pellet was then resuspended in 20 ml PBS and homogenised on top speed for one minute to release *L. intracellularis* bacteria.

This homogenate was centrifuged at 1000 xg for 4 minutes giving a pellet containing a crude mixture of homogenised epithelial cells and intestinal bacteria. The supernatant was filtered using filters with pore sized 3 mm, 1.2 mm and 0.8 mm (Millipore Corporation, MA, USA). The filtrate was centrifuged at 8000 xg for 30 minutes, resulting in a small pellet of *L. intracellularis* bacteria. The *L. intracellularis* bacteria were further purified using a 45% self forming percoll gradient as follows: 2 mls of the bacterial preparation was mixed by inversion into 30 mls of a 45% self forming Percoll (Pharmacia LKB, Uppsala, Sweden) gradient (45% v/v of Percoll, 150 mM NaCl). The gradients were centrifuged in a Sorval centrifuge using the SS34 rotor, at 20,000rpm for 30 minutes at 4°C. Usually a number of bands form within the gradient. The band (usually located approx. 10-20mm from the base of the tube) containing the *L. intracellularis* bacteria was collected and the volume made up to 16 mls with PBS. The solution was then centrifuged for 15 minutes at 8000rpm. The resultant pellet was washed with PBS before being resuspended in a final volume of approximately one ml.

EXAMPLE 3

PURIFICATION OF *LAWSONIA INTRACELLULARIS* GENOMIC DNA

Lawsonia intracellularis genomic DNA was purified from scrapings taken from normal and infected pig ilea using a percoll gradient purification method described by Anderson *et al* (11) and Sambrook *et al* (12).

EXAMPLE 4

IMMUNOSCREENING OF GENOMIC LIBRARIES

A lambda ZAP *L. intracellularis* genomic library was plated on a lawn of *Escherichia coli* BB4 (23) cells at a density of 10,000 plaque-forming units (pfu) per 150 mm L-broth agar plate. The library was screened with a rabbit or pig anti- *L. intracellularis* sera using the method described in the Protoblot Technical Manual (Promega, WI, USA). Filters were blocked in a buffer containing 10mM Tris HCl, pH8.0, 150mM NaCl, 0.05% Tween 20, 1% w/w gelatin. Positive plaques identified in a primary screen were picked, replated at a lower density and rescreened until individual positive plaques

were identified.

EXAMPLE 5

ISOLATION AND SEQUENCING OF cDNA INSERTS

Phagemid DNA from positive λZAP phage clones was isolated by excision in vivo of the pBluescript phagemid under the conditions recommended by Stratagene (CA, USA). Plasmid DNA was extracted by the method of Birnboim and Doly (21). DNA sequencing of cDNA inserts was performed by the chain termination method (22).

EXAMPLE 6

ANTISERA

Antisera to *L. intracellularis* bacteria were raised in rabbits and pigs. Rabbits were injected intramuscularly with a preparation of percoll gradient-purified *L. intracellularis* bacteria mixed with a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, CSL Limited, Melbourne, Australia), and then with Tween 80 enhancer. Two 3 ml injections, containing 9 mg protein, were given four weeks apart. Blood samples were collected from the ear vein prior to immunisation and two weeks following the second injection.

A 6-week old pig was hyperimmunised by intramuscular injection of percoll gradient purified *L. intracellularis* bacteria prepared with Freund's incomplete adjuvant as for the rabbit. Three injections of the prepared antigen were administered four weeks apart, and blood was collected from the vena cava vein two weeks following the final injection. Serum samples were also collected from six 4-week old pigs prior to challenge with *L. intracellularis* and three weeks post challenge when pigs were shedding *L. intracellularis* bacteria in their faeces. Diluted pig sera (1 ml, 1 in 1000) were pre-absorbed with 100 ml *E. coli* DH5α (24) lysate for 1 h at room temperature with gentle mixing. The lysate was prepared by freeze-thawing a suspension of *E. coli* in PBS.

EXAMPLE 7

SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Protein samples were resuspended in 250 ml of sample buffer (62.4 mM HCl, 2% w/v SDS, 10% v/v glycerol, 5% v/v 20 mercaptoethanol, 0.002% bromophenol blue, pH 6.8) and heated to 95°C for 5 minutes before separating solubilised proteins electrophoretically on a 0.1% w/v SDS-12% w/v PAGE vertical slab gel (13).

EXAMPLE 8

WESTERN BLOTTING

Proteins were electrophoretically transferred to Immobilon-P (Millipore Corporation, MA, USA) membranes in a Trans-Blot Cell (BioRad, CA, USA) at 100 V for 1 h in a buffer containing CAPS (3-[Cyclohexylamino]-l-propanesulfonic acid, pH 11, Sigma, MI, USA) and 10% v/v methanol. The membranes were then blocked with 10% w/v Blotto (Diploma skim milk powder, Melbourne, Australia) in PBS containing 0.05% v/v Tween-20 (PBST) for 1 h at room temperature with gentle rocking. The filters were washed once in 0.1% w/v Blotto, PBST (50 ml) for 5 min, and transferred to antisera diluted in 1% w/v Blotto, PBST. Rabbit antisera was diluted 1 in 2000 and pre-absorbed pig antisera was diluted 1 in 1000. The filters were incubated in either rabbit or pig antisera for 1 h followed by washing twice as before.

Peroxidase-conjugated goat anti-rabbit immunoglobulins (Silenus, Melbourne, Australia), diluted 1:3000 in 1% w/v Blotto, PBST were used to detect rabbit antibodies. Simiarly, HRP conjugated anti-swine immunoglobulins (DAKO, CA, USA) were applied at a dilution of 1:1000. Enhanced Chemiluminescence (ECL, Amersham, IL, USA) was used to discriminate *L. intracellularis* proteins. Prior to ECL detection, blots were washed as before for 1 h, changing the wash solution every 15 min. The filters were exposed to autoradiographic film (Agfa, NJ, USA) for less than 1 minute before developing.

EXAMPLE 9

PRODUCTION OF RECOMBINANT GroEL

Clones found to be positive according to the immunoscreening method described in Example 4 were sequenced using the protocol detailed in Example 5. One clone isolated represented the GroEL protein. The nucleotide [SEQ ID NO:1] and corresponding amino acid [SEQ ID NO:2] of GroEL are shown in Figure 1. Another clone isolated represented the GroES protein. The nucleotide sequence of GroES [SEQ ID NO:3] is shown in Figure 2. The corresponding amino acid sequence is defined in SEQ ID NO:4. The term "rGroEL" and "rGroES" refer to recombinant forms of GroEL and GroES, respectively.

The coding region of *L. intracellularis* GroEL was amplified using the following primers; Primer one: CGAGGATCCGATGGCTTCTAAAGAAATCC (SEQ ID NO:5) and Primer two: GGAATTCGGATCCCTAGTACATACCGTCCAT (SEQ ID NO:6). The resulting PCR product was digested with *Bam*HI and cloned into *Bam*HI site of pBS. The resulting clone was called pBS-EL2. The *Bam*HI insert of pBS-EL2 was then cloned into pET15b. The resulting clone which contained the GroEL transcript in the correct orientation was named pET-EL2. An overnight culture of *E. coli* BL21 (25) transformed with pET-EL2 was diluted 1:10 in L-broth ampicillin, 1 mM IPTG and grown at 37°C with shaking at 200rpm for 2 hours. The bacterial pellet was recovered by centrifugation and the bacteria were then lysed by a freeze thaw cycle. Recombinant GroEL protein was purified under denaturing conditions over a nickel charged column using the conditions recommended by the manufacturer (Novagen, WI, USA). Purified protein was confirmed to be GroEL by SDS-PAGE followed by Western analysis, as described in Examples 7 and 8 using a rabbit anti *L. intracellularis* sera. The purified recombinant GroEL was dialysed against PBS and then repurified by nickel charged column chromatograghy under non-denaturing conditions. A centricon 10 (Amicn, MA, USA) column was then used to buffer exchange into PBS and concentrate the protein.

EXAMPLE 10

CONSTRUCTION OF pCIGH-EL2

The coding region of GroEL was excised from pBS-EL2 and cloned into the *Bam*HI site of the DNA vaccine vector, pCIGH. In this construct GroEL expression is directed by the CMV promoter. pCIGH containing *L. intracellularis* GroEL in the correct orientation was named pCIGH-EL2. To confirm that pCIGH-EL2 correctly encoded for the expression of *L. intracellularis* GroEL, pCIGH-EL2 was transfected into COS cells using standard techniques and expression of GroEL protein was confirmed by SDS-PAGE followed by western analysis, described in Example 8, using the rabbit anti *L. intracellularis* sera (Example 6). *Escherichia coli* carrying pCIGH-EL2 was deposited at the Australian Government Analytical Laboratories, Pymble, New South Wales, 2073, Australia on 28 November, 1995 under Accession Number N95/72592.

EXAMPLE 11

PRODUCTION OF pCIGHEL2 DNA FOR VACCINATION

For vaccine immunisations pCIGH-EL2 was purified in the following manner. *E. coli* DH5α cells transformed with pCIGH-EL2 were grown overnight at 37°C with shaking in one litre LB-broth cultures containing ampicillin. The bacterial pellet was recovered by centrifugation at 10,000 g for 10 minutes. The plasmid DNA was then purified by alkaline lysis (21). Endotoxin was removed by triton X-114 phase extraction as described by Manthorpe et al (19).

EXAMPLE 12

rGroEL ELISA

Recombinant GroEL at a concentration of 1-5ug/ml in PBS was incubated in the wells of a microtitre plate overnight at 4°C. The wells were then blocked by the addition of 5% W/V blotto for one hour at room temperature. The wells were then washed with PBST three times before serial dilutions of pig sera was added to each well. Following

a one hour incubation at room temperature the wells were washed three times with PBST before 50 ul of a 1:3000 dilution of goat anti pig immunoglobulins conjugated with horse radish peroxidase (DAKO, CA, USA) was added. Following a one hour room temperature incubation the wells were washed three times with PBST before the HRP substrate was added. The colour was allowed to developed for 30 minutes before the optical density of each well was read at 414 nm using a Titretek Multiscan MCC.

EXAMPLE 13

T-CELL RESPONSES TO rGroEL

Peripheral blood lymphocytes were purified from 10 mls of pig blood collected in the presence of lithium heparin by the following method. The whole blood samples were centrifuged at room temperature for 10 minutes at 2000 rpm and the buffy coat cells collected. This cell mixture, approximately 2 ml, was diluted to 4 mls by the addition of PBS. The cell mixture was then overlayed on 4 mls of ficoll-paque (Pharmacia, Uppsala, Sweden) and the tubes centrifuged at room temperature for 20 minutes at 2000 rpm. The resulting band containing the lymphocytes was recovered. The lymphocytes were washed in PBS before being resuspended in RPMI (Gibco-BRL, MD, USA) media at a concentration of 2×10^6/ml. An amount of 100 ul of this suspension was added to each well of a microtitre plate containing 100 ul RPMI, 20% w/v FCS, 100 units/ml Penicillin, 0.1 mg/ml Steptomycin, 2mM L-Glutamine. To each test well 1 ug of rGroEL was added. The controls used were as follows: positive control Con A 5ug/ml, negative control GST from *F. hepatica* at 1 ug/well. The microtitre plate was incubated at 37°C 10% v/v $C0_2$ for 72 hours before 1 uCi of Methly-^{3}H Thymidine (Amersham, IL, USA) (46Ci/mmol) was added to each well. Following a further 9 hour incubation the cells were harvested using a Skatron Harvester and the amount of radioactivity of each well determined by counting in a Packcard Matrix 9600 Betacounter.

EXAMPLE 14

IMMUNOFLORESCENT DETECTION OF *LAWSONIA INTRACELLULARIS* BACTERIA IN PIG FAECES

Faecal swabs of pigs were taken using a cotton tipped swab and then the sample was smeared onto a glass slide. After allowing ten minutes for air drying the smears were heat fixed by heating to 60°C for approximately 10 seconds. The slides were then rinsed in PBS. An amount of 30ul of a 1/200 dilution of a mouse ascites containing IG4 monoclonal antibody (see Example 1) was added, a glass cover slip applied, and the slides were incubated at room temperature for 40 minutes. The cover slip was removed and the slides were washed (PBST for 7 minutes, three times). An amount of 30ul of a 1/40 dilution of a FITC conjugated anti-mouse antiserum (Silenus, Melbourne Australia) was added, a glass cover slip applied and the slides were incubated at room temperature for 40 minutes. The cover slip was removed and the slides were washed (PBST X3 for 7 minutes). The slides were given a final rinse in PBS. A drop of 10% v/v glycerol PBS was added and a glass cover slip applied. The fluorescent bacteria were visualised under highpower (X1200) at 340 nm using a Lietz laborlux S microscope. Twenty fields were counted and the results (see Table 1) were expressed as the average number of *L. intracellularis* bacteria per high powered field.

EXAMPLE 15

FORMALIN-KILLED *L. INTRACELLULARIS VACCINE*

The percoll gradient purified bacterial *L. intracellularis* pellet was resuspended in 1 ml of 1% formal saline and incubated overnight at 4°C. The percoll gradient-purified *L. intracellularis* bacteria was then mixed into a double-emulsion made by processing with oil adjuvant (Freund's incomplete adjuvant, Commonwealth Serum Laboratories, Melbourne, Australia), and then with Tween 80 enhancer.

EXAMPLE 16

VACCINATION PROTOCOL

Sixteen weaned pigs (Landrace crossed with Large White) were sourced from a Pig Improvement Company piggery and treated with Neo- Terramycin (0.25 g/kilo) for 5 days. Seven days later (day -40) pigs Y1-Y8, Y10, Y12, Y14 and Y16 were vaccinated as described. Pigs Y3, Y6, Y11 and Y13 were treated for abscess with long acting terramycin on day -34.

The sixteen pigs were divided into four groups and treated as follows:

Group 1 Negative Control Group

Four pigs (Ear Tag No. Y1-Y4) were immunised with 200 μg of pCIGH in 200 μl of BBS intramuscularly on days -40, -26 and -12.

Group 2 GroEL DNA Vaccine

Four pigs (Ear Tag No. Y5-Y8) were immunised with 200 μg of pCIGH-EL2 DNA intramuscularly on days -40, -26 and -12.

Group 3 Whole Bacteria Vaccine

Four pigs (Ear Tag No. Y10, Y12, Y14 and Y16) were immunised with formalin killed *L. intracellularis* bacteria emulisifed in 0.5 ml of PBS/Freunds incomplete on days -33 and -12.

Group 4 Uninfected Controls

Four pigs (Ear Tag No. Y9, Y11, Y13 and Y15) received no treatments and were housed in a separate area from the vaccinated and orally challenged pigs.

The results of the vaccination are shown in Table 1.

EXAMPLE 17

ORAL CHALLENGES OF INFECTED PIGS

Infected ilea were collected from pigs vaccinated as described in Example 16 and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 150g of infected mucosa was scraped from the intestinal wall. The mucosa was homogenised with an equal volume of sterile PBS on half speed for 20 s using a Sorvall ominimixer. This suspension was diluted two fold with sterile PBS to form the challenge suspension.

On day 0 each pig from Groups 1-3 was dosed with a 5% Na Bicarb solution (10 ml/kg) followed by 30 ml of the challenge suspension. This was repeated on day 1 and day 2.

From day 11 onwards, the number of *L. intracellularis* bacteria in each pig's faeces was monitored by immunoflorescence. Pigs were monitored for signs of disease and shedding of *L intracellularis* bacteria. Pigs shedding greater than 100 bacteria per high powered field and scouring were killed for ethical reasons.

On day 22 the surviving pigs were humanely killed and the small intestines were recovered. Two sections of small intestine were removed 5 cms and 17 cms proximally from the ileocaecal junction. These sections were fixed in 10% v/v formalin, wax embedded and sections were sent to an independent veterinary pathologist for analysis.

EXAMPLE 18

LAWSONIA INTRACELLULARIS PROTEINS RECOGNISED BY VACCINATED PIGS

Antibodies raised to *L. intracellularis* proteins post vaccination by pigs were analysed by Western blotting followed by ECL (Amersham, IL, USA) detection as described in Example 8. The results are shown in Figure 3. Vaccinated pigs produce antibodies to a range *of L. intracellularis* proteins. The most immunodominant proteins recognised are approximately 62.7 Kda, 58.7 Kda, 57.2 Kda, 44 Kda, 36.7 Kda and two smears from

24-26 Kda and 22-23.5 Kda. Minor immunoreactive bands had approximately the following molecular weights 67 Kda, 52.5 Kda, 50.5 Kda, 50 Kda, 48.2 KDa, 47.9 Kda, 44.7 Kda, 43.5 Kda, 42.5 Kda, 41.5 Kda, 40.5 Kda, 39 Kda, 35.3 Kda, 17 Kda, 15.5 Kda, 12 Kda and 7 Kda. The molecule weight of the proteins recognised will vary by up to 5% depending on the method used for estimation.

EXAMPLE 19

SHEDDING OF *L. INTRACELLULARIS* BACTERIA BY PIGS DURING THE TRIAL

Three of the pigs vaccinated with vector alone (Group 1 Nos. Y1, Y2 and Y4) shed greater than 100 *L. intracellularis* bacteria per high powered field in their faeces by day 19 post oral challenge (Table 1). Two of these pig (Y2 and Y4) had a bloody scour. All three pigs were humanely killed on day 20. Y3 shed low levels of *L. intracellularis* bacteria during the course of the infection trial. Maximal bacterial shedding for Y3 was 16 bacteria per high powered field.

All pigs in group 2 vaccinated intramuscularly with 200 ug of the pCIGH-EL2 DNA shed *L. intracellularis* bacteria. The amount of bacterial shedding was much reduced relative to the pigs in group 1. Maximal shedding was 10, 3, 15 and 2 *L. intracellularis* bacteria per high powered field for pigs Y5-Y8 respectively.

All pigs in groups 1-3 shed *L. intracellularis* bacteria during the course of the trial. However, pigs in group 3 (formalin -killed *L. intracellularis* vaccine) never shed more than 3 *L. intracellularis* bacteria per high powered field. Vaccination with the formalin killed *L. intracellularis* vaccine reduced total bacterial shedding of *L. intracellularis* bacteria by vaccinated pigs by 98.5% when compared with group 1 pigs.

None of the group 4 pigs (uninfected controls) shed any *L. intracellularis* bacteria during the course of the trial.

EXAMPLE 20

GROSS PATHOLOGY

Group 1 *-ve control group*

Y1 Approximately 5 cm of terminal ileum was grossly thickened. No other signs of PPE were evident macroscopically. Findings are consist with intestinal adenomatosis (See Figure 4).

Y2 The intestine was found to be grossly thickened and the serosa had the characteristic cerebriform forms (Figure 5). Over 2.5 metres of the intestine was involved. The lumen of the intestine was found to contain fresh blood and fibrinous casts were evident. Proliferative haemorrhagic enteropathy.

Y3 No gross signs of PPE were evident.

Y4 The intestine was found to have necrotic enteritis (Figure 6). The mucosal surface was replaced with a fibrinous pseudomembrane. Oedema of the mesentery was clearly evident. Over 2.0 meters of intestine was involved.

Group 2 *GroEL DNA vaccine*

Y5 No gross signs of PPE.

Y6 Possible thickening of the mucosa.

Y7 No gross signs of PPE.

Y8 No gross signs of PPE.

Group 3 *Whole L. intracellularis cell vaccine*

Y10 No gross signs of PPE.

Y12 No gross signs of PPE.

Y14 No gross signs of PPE.

Y16 No gross signs of PPE.

Group 4 *Uninfected controls*

Y9 No gross signs of PPE.

Y11 No gross signs of PPE.

Y13 No gross signs of PPE.

Y15 No gross signs of PPE.

EXAMPLE 21

HISTOPATHOLOGY REPORT

Reports are based on established histopathological descriptions in Jubb, *et al* (20).

Group 1 -ve control group

Y1 Numerous microfocal/confluent lesions of Porcine Intestinal Adenomatosis (PIA) are associated with Peyers Patches.

Y2 Serious generalised (annular) lesions of Porcine Intestinal Adenomatosis.

Y3 No conclusive evidence of PIA. Sparse microfocal lesions suggestive of a non-specific mild reactive (reparational) hyperplasia (rather than an adenomatosis).

Y4 Severe generalised (annular) lesions of PIA.

Group 2 GroEL DNA vaccine

Y5 The 5cm section had marked generalised PIA associated with Peyers Patches: the remaining portions of this piece of mucosa have microfocal lesions of PIA. At 20 cm generalised microfocal PIA.

Y6 No conclusive evidence of PIA.

Y7 A single tiny microfocus of probable PIA is associated with Peyers Patch

Y8 No conclusive evidence of PIA.

Group 3 Whole L. intracellularis cell vaccine

Y10 No conclusive evidence of PIA.

Y12 No conclusive evidence of PIA.

Y14 No conclusive evidence of PIA.

Y16 No conclusive evidence of PIA. Possible single microfocus of PIA is associated with Peyers Patch.

Group 4 *Uninfected controls*

Y11 No conclusive evidence of PIA.

Y9 No conclusive evidence of PIA.

Y13 Intestine was not recovered since pig was killed due to lameness at day 15.

Y15 Diagnosis not possible because of the poor quality of the section.

EXAMPLE 22

ANTIBODY RESPONSES TO GroEL INDUCED BY VACCINATION

Antibody responses to GroEL were measured according to the method described in Example 12 and results are shown in Figure 7. All pigs vaccinated with pCIGH-EL2 DNA produced detectable antibodies to the GroEL protein by day 2. By day 17 pigs Y5 -Y8 had end titres of 1:1,600. No other pigs had detectable antibody to GroEL protein, except Y1 and Y2 which had titres that were just detectable.

EXAMPLE 23

T-CELL RESPONSES TO GroEL INDUCED BY VACCINATION

T-Cell response was determined according to the method described in Example 13. T-Cell proliferation in response to both GroEL and GST protein are shown in Figure 8. Peripheral blood lymphocytes purified from pigs Y5-Y8 on day 2, proliferated in response to exposure to GroEL protein. On average this response was approximately three times that seen with the negative control antigen, GST.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

TABLE 1

	Vaccination			Challange																		
	Day -40	Day -33	Day -28	Day -14	Day 0	Day 1	Day 2	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22
1	pCIGH	200ug in PBS	200ug in PBS	200ug in PBS				1+	1+	0	0	5+	10+	50+	100+	15	5 cm of thickening		
2	pCIGH	200ug in PBS	200ug in PBS	200ug in PBS				0	1+	1+	1+	3+	1+	70+	100+	100	PHE 2 5 M		
3	pCIGH	200ug in PBS	200ug in PBS	200ug in PBS				0	0	0	0	0	0	1+	4	16	1+	0	1
4	pCIGH	200ug in PBS	200ug in PBS	200ug in PBS				1+	0	0	10+	0	5+	60+	200+	80	PHE 2.0 M		
5	pCIGH-EL2	200ug in PBS	200ug in PBS	200ug in PBS				0	0	0	0	0	0	5+	8	10	2	10	6
6	pCIGH-EL2	200ug in PBS	200ug in PBS	200ug in PBS				0	0	0	0	0	0	1+	3	1	0	0	0
7	pCIGH-EL2	200ug in PBS	200ug in PBS	200ug in PBS				0	0	1+	0	0	3+	0	15	1	0	0	0
8	pCIGH-EL2	200ug in PBS	200ug in PBS	200ug in PBS				0	0	1+	0	0	0	1+	2	1	0	2	0
9	pCIGH-EL2	200ug in PBS	200ug in PBS	200ug in PBS				0	0	0	0	0	1+	1+	0	0	0	0	0
10	Whole bugs		1ml killed whole cell	1ml killed whole cell				0	0	0	0	0	2+	0	0	0	0	0	0
12	Whole bugs		1ml killed whole cell	1ml killed whole cell				1+	0	0	0	0	1+	0	<1	<1	0	0	0
14	Whole bugs		1ml killed whole cell	1ml killed whole cell				0	0	0	0	0	0	0	3	<1	0	0	0
16	Whole bugs		1ml killed whole cell	1ml killed whole cell				0	0	0	0	0	0	0	0	0	0	0	0
9	Uninfected controls							0	0	0	0	0	0	0	0	0	0	0	0
11	Uninfected controls							0	0	0	0	Killed Lama							
13	Uninfected controls							0	0	0	0	0	0	0	0	0	0	0	0
15	Uninfected controls																		

REFERENCES:

1 Baker, I.K. and Van Dreumel, A.A. (1985) In "Pathology of Domestic Animals," 3rd Edition, Vol. 2 p. 1-237, eds K.V.F. Jubb, P.C. Kennedy and N. Palmer. (Academic Press: Orlando).

2 Rowland, A.C. and Lawson, G.H.K. (1976) *Veterinary Record* **97**:178-180.

3 Love, R.J. and Love, D.M. (1977) *Veterinary Record* **100**:473

4. Johnsson, L. and Martinsson, K. (1976) *Acta Veterinaria Scandinavica* **17**:223-232.

5. O'Neil, P.A. (1970) *Veterinary Record* **87**:742-747.

6. Straw, B.E. (1990). *Journal of American Veterinary Medical Association* **197**: 355-357.

7. Gebhert, C.J., Barns, S.M., McOrist, S., Lin, G.F., Lawson, G.H.K, (1993) *International Journal of Systemic Bacteriology* **43**: 433-438.

8. Gebhert, C.J., Ward, G.E., Chang, K. And Kurtz, H.J. (1983). *American Journal of Veterinary Research* **44**:361-367.

9. Lawson, G.H.K., McOrist, S., Jansi, S. and Mackie, R.A. (1993) *Journal of Clinical Microbiology* **31**:1136-1142.

10. McOrist, S., Boid, R., Lawson, G.H.K. and McConnell, I. (1987) *The Veterinary Record* **121**:421-422.

11. Anderson, B.J., M.M. Bills, J.R. Egerton, and J.S. Mattick. (1984) *Journal of Bacteriology* **160**:748-754.

12. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning. A laboratory manual. Second edition. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y.

13. Laemmli, U.K. (1970) *Nature* **227:** 680-685.

14. McOrist, S, Jasni, S, Mackie, RA, MacIntyre, N, Neef, N. and Lawson GHK (1993) *Infection and Immmunity* **61**: 4286-4292.

15. Fox, JG, Murphy, JC, Otto, G Pecquet-Goad, ME, Larson, QHK and Scott JA (1989) *Veterinary Pathology* **26:** 515-517.

16. Elwell, MR, Chapman, AL and Frenkel, JK (1981) *Veterinary Pathology* **18:** 136-139.

17. Schodeb, TR and Fox JG (1990) *Veterinary Pathology* **27:** 73-80.

18. Mason, RW, Monkton, P and Hasse D (1995) *Australian Veterinary Journal* (in press).

19. Manthorpe, M, Cornefert-Jensen, F., Hartikka, J., Felgner, J, Rundell, A, Margalith, M and Dwarki, V. (1993) *Human Gene Therapy* **4:** 419-431.

20. Jubb KVC, Kennedy, PC and Palmer, NC (1993). The Pathology of Domestic Animals 4th ed. San Diego, CA, Academic Press pp 229-233.

21. Birnboim, HC and Doly J (1979) *Nucleic Acids Research* **7:** 1513.

22. Sanger, F, Nicklen, S and Coulson, AR (1977) *Proceedings of the National Academy of Science* **74:** 5463.

23. Block, WO, Fernandes, JM and Short, JM (1987) *Biotechnics* **5:** 376-79.

24. Woodcock, DM *et al* (1989) *Nucleic Acids Research* **17:** 3469-78.

25. Studier, FW *et al* (1990) *Methods in Enzymology* **185:** 60-89.

26. McOrist, S *et al* (1995) *International Journal of Systematic Bacteriology* **45:** 820-825.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: THERAPEUTIC AND DIAGNOSTIC COMPOSITIONS - A

(iii) NUMBER OF SEQUENCES: 7

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 759 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..759

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Ala Ser Lys Glu Ile Leu Phe Asp Ala Lys Ala Arg Glu Lys Leu
 1               5                  10                  15

TCA CGA GGT GTA GAT AAA CTT GCA AAT GCT GTT AAA GTA ACA CTT GGA      96
Ser Arg Gly Val Asp Lys Leu Ala Asn Ala Val Lys Val Thr Leu Gly
            20                  25                  30

CCT AAA GGC CGT AAT GTC GTT ATT GAA AAG TCT TTT GGT TCC CCA GTT     144
Pro Lys Gly Arg Asn Val Val Ile Glu Lys Ser Phe Gly Ser Pro Val
        35                  40                  45

ATT ACA AAA GAT GGT GTA TCT GTT GCA AAA GAA ATT GAA CTT GAA GAT     192
Ile Thr Lys Asp Gly Val Ser Val Ala Lys Glu Ile Glu Leu Glu Asp
    50                  55                  60

AAG TTT GAA AAT ATG GGC GCT CAA ATG GTT AAA GAA GTA GCT CCC AAA     240
Lys Phe Glu Asn Met Gly Ala Gln Met Val Lys Glu Val Ala Pro Lys
65                  70                  75                  80

ACT AGC GAT ATT GCT GGT GAT GGA ACT ACA ACA GCA ACA GTC CTT GCA     288
Thr Ser Asp Ile Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala
                85                  90                  95

CAA GCT ATT TAT CGT GAA GGT GTA AAA CTT GTA GCA GCT GGT CGT AAT     336
Gln Ala Ile Tyr Arg Glu Gly Val Lys Leu Val Ala Ala Gly Arg Asn
            100                 105                 110

CCT ATG GCC ATT AAA CGT GGC ATA GAT AAA GCT GTT GTT GCT GTT ACT     384
Pro Met Ala Ile Lys Arg Gly Ile Asp Lys Ala Val Val Ala Val Thr
        115                 120                 125

AAA GAA CTA AGC GAC ATT ACA AAG CCT ACT CGT GAC CAA AAA GAA ATA     432
Lys Glu Leu Ser Asp Ile Thr Lys Pro Thr Arg Asp Gln Lys Glu Ile
    130                 135                 140
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GCT CAA GTT GGA ACC ATT TCT GCA AAC TCT GAT ACA ACA ATA GGT AAT      480
Ala Gln Val Gly Thr Ile Ser Ala Asn Ser Asp Thr Thr Ile Gly Asn
145                 150                 155                 160

ATC ATA GCT GAA GCT ATG GCT AAA GTT GGA AAA GGA GGT GTT ATC ACA      528
Ile Ile Ala Glu Ala Met Ala Lys Val Gly Lys Gly Gly Val Ile Thr
                165                 170                 175

GTT GAG GAA GCT AAA GGT CTT GAA ACT ACA TTA GAT GTG GTT GAA GGA      576
Val Glu Glu Ala Lys Gly Leu Glu Thr Thr Leu Asp Val Val Glu Gly
            180                 185                 190

ATG AAG TTT GAC CGT GGC TAC CTC TCT CCA TAC TTT GTA ACT AAT CCT      624
Met Lys Phe Asp Arg Gly Tyr Leu Ser Pro Tyr Phe Val Thr Asn Pro
        195                 200                 205

GAG AAA ATG GTT TGT GAA CTT GAT AAC CCT TAT ATC CTT TGT AAT GAG      672
Glu Lys Met Val Cys Glu Leu Asp Asn Pro Tyr Ile Leu Cys Asn Glu
    210                 215                 220

AAA AAG ATT ACT AGC ATG AAA GAC ATG CTA CCA ATC TTA GAA CAA GTT      720
Lys Lys Ile Thr Ser Met Lys Asp Met Leu Pro Ile Leu Glu Gln Val
225                 230                 235                 240

GCT AAA GTA AAC CGT CCA CTC CTT ATT ATT GCT GAA GAC                  759
Ala Lys Val Asn Arg Pro Leu Leu Ile Ile Ala Glu Asp
                245                 250
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 253 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 1               5                  10                  15

Ser Arg Gly Val Asp Lys Leu Ala Asn Ala Val Lys Val Thr Leu Gly
            20                  25                  30

Pro Lys Gly Arg Asn Val Val Ile Glu Lys Ser Phe Gly Ser Pro Val
        35                  40                  45

Ile Thr Lys Asp Gly Val Ser Val Ala Lys Glu Ile Glu Leu Glu Asp
    50                  55                  60

Lys Phe Glu Asn Met Gly Ala Gln Met Val Lys Glu Val Ala Pro Lys
65                  70                  75                  80
```

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Thr Ser Asp Ile Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala
                  85                  90                  95

Gln Ala Ile Tyr Arg Glu Gly Val Lys Leu Val Ala Ala Gly Arg Asn
             100                 105                 110

Pro Met Ala Ile Lys Arg Gly Ile Asp Lys Ala Val Val Ala Val Thr
         115                 120                 125

Lys Glu Leu Ser Asp Ile Thr Lys Pro Thr Arg Asp Gln Lys Glu Ile
     130                 135                 140

Ala Gln Val Gly Thr Ile Ser Ala Asn Ser Asp Thr Thr Ile Gly Asn
145                 150                 155                 160

Ile Ile Ala Glu Ala Met Ala Lys Val Gly Lys Gly Gly Val Ile Thr
                165                 170                 175

Val Glu Glu Ala Lys Gly Leu Glu Thr Thr Leu Asp Val Val Glu Gly
             180                 185                 190

Met Lys Phe Asp Arg Gly Tyr Leu Ser Pro Tyr Phe Val Thr Asn Pro
         195                 200                 205

Glu Lys Met Val Cys Glu Leu Asp Asn Pro Tyr Ile Leu Cys Asn Glu
     210                 215                 220

Lys Lys Ile Thr Ser Met Lys Asp Met Leu Pro Ile Leu Glu Gln Val
225                 230                 235                 240

Ala Lys Val Asn Arg Pro Leu Leu Ile Ile Ala Glu Asp
                245                 250
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 306 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..303

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG AAC CTG AAA CCT TTG AAT GAC CGT GTT TTA GTA AAA CGT CTT GAA      48
Met Asn Leu Lys Pro Leu Asn Asp Arg Val Leu Val Lys Arg Leu Glu
 1               5                  10                  15

TCT GAA GAA AAA ACA GCT GGT GGA CTC TAT ATC CCT GAT ACT GCT AAA      96
Ser Glu Glu Lys Thr Ala Gly Gly Leu Tyr Ile Pro Asp Thr Ala Lys
            20                  25                  30

GAA AAA CCA TCT CGT GGT GAA GTT GTT GCT GTT GGA CCT GGT AAA CAT     144
Glu Lys Pro Ser Arg Gly Glu Val Val Ala Val Gly Pro Gly Lys His
        35                  40                  45

ACA GAT GAT GGT AAA TTA ATA CCT ATG GCT GTA AAA GCA GGA GAT ACA     192
Thr Asp Asp Gly Lys Leu Ile Pro Met Ala Val Lys Ala Gly Asp Thr
    50                  55                  60

GTT CTT TTT AAT AAG TAT GCA GGA ACA GAA GTA AAG CTT GAT GGT GTA     240
Val Leu Phe Asn Lys Tyr Ala Gly Thr Glu Val Lys Leu Asp Gly Val
65                  70                  75                  80

GAG CAT CTA GTT ATG CGT GAA GAT GAC ATC CTA GCT GTT ATT ACT GGA     288
Glu His Leu Val Met Arg Glu Asp Asp Ile Leu Ala Val Ile Thr Gly
                85                  90                  95

GAA ACT GGC CGC AAG TGA                                            297
Glu Thr Gly Arg Lys  *
            100
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Asn Leu Lys Pro Leu Asn Asp Arg Val Leu Val Lys Arg Leu Glu
 1               5                  10                  15

Ser Glu Glu Lys Thr Ala Gly Gly Leu Tyr Ile Pro Asp Thr Ala Lys
            20                  25                  30

Glu Lys Pro Ser Arg Gly Glu Val Val Ala Val Gly Pro Gly Lys His
        35                  40                  45

Thr Asp Asp Gly Lys Leu Ile Pro Met Ala Val Lys Ala Gly Asp Thr
    50                  55                  60

Val Leu Phe Asn Lys Tyr Ala Gly Thr Glu Val Lys Leu Asp Gly Val
65                  70                  75                  80

Glu His Leu Val Met Arg Glu Asp Asp Ile Leu Ala Val Ile Thr Gly
                85                  90                  95

Glu Thr Gly Arg Lys
            100
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CGAGGATCCG ATGGCTTCTA AAGAAATCC                                    29
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAATTCGGA TCCCTAGTAC ATACCGTCCA T 31

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4972 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
   1  AACTCCTGGT CTATCAAGAT CAACTAAAAA ATATTCTTTA TCTAATAGTT
  51  GCTCAAAAAT AATTGTACCT ACAGGTAAAT GAAGAATCAA ATCTTCCCCT
 101  TTTTTACCAT GACGCTGGCT CCCTTTACCA CCTTCTCCAT TTTGAGCTCT
 151  ATAGTGACGT TGCACACGAA AATCATAAAG GGTTAACAAA CGTGAATCAG
 201  CTTTAAAAAT TATATTACCT CCATCTCCTC CATCCCCTCC ATTAGGTCCA
 251  CCTTTAGGTA TAAACTTTTC GCGTCTAAAT GAAACACATC CATTTCCACC
 301  TTTTCCTGCG CTCACGCTAA TAGTTACTTC ATCAACAAAA CGCATGATTA
 351  TCCTTTCAAT AACAAATATC TATTCAATAC TGTTACTAAC TTGTTTACTG
 401  TTTTTTCTAG AAAATTACCT GGCTAATTAT TATAGTTATA TCTAGATTAA
 451  TGAAAAAGGA AGAAGTCATT ACACTCCTTC CTTATTAATA GAATCCTGGA
 501  ATAATTATTA TACGGTGGGT TGTATATGCA CTCTACTATA TCTTTTACAT
 551  TTACGAAAAT ATGTTTCATA AGTTACTATA CCATTAACTT TTGCAAATAA
 601  AGTATAGTCT CTTCCCATTC CAACATTTTC TCCAGGATGA ATTTTTGTAC
 651  CTAGTTGACG AACAAGGATA TTGCCTGCCA AGACTTTCTG GCCGCCGAAA
 701  CGCTTTATAC CACGACGTTG TCCTGGACTA TCTCTACCAT TGCGAGAACT
 751  TCCACCAGCT TTCTTATGGG CCATTTTAAT ATCTCCTTAA AGCTGAATAC
 801  CTGTTACTTT TAGAGCTGTA TAGTCTTGAC GATGACCTTG GAGTTTACGT
 851  GAGTCATTTC TTCTCCACTT TTTAAAAACA AGAATTTTTT TATCACGACC
 901  ATGCTCAAGA ACTTTAGCTA TAACTTTAGC ATTATTAATA TATGGTGTTC
 951  CAATTTGAGG AGATGAACCA CCAATCATAA AAATTTTATC AAAAAAAATT
1001  TCTGTTCCAA CTTCAGCGTC TATTTTAGAA ACAAAAATTT TAGAACCCTC
1051  TTCAACACAG AATTGTTTTC CACCAGCTTC AATAATTGCG TACATAAATA
1101  ATGTGCCTCC CAAAAAAGAC AAGAAATACT AATTTGATAT TTTCAATATT
1151  GTCAAGTAGG AACTTTATCT TTAGAATGTT AGATGTAACA ATTTTTTTAG
```

```
1201  AAAAAAAATA TTTTCAATAC AATAGGAAAA GAGGAAAAAA AAAAAGATTT
1251  TTAGAAAAAA TTTTTATTTC TCCAAAAAAT GCAAAAATAT AAAAAATTCT
1301  AATAGGATAG AAGTTATTAC TGTATTGATT TTCAAGACTT ACTTAAAAAT
1351  TTTTATAAAA AAATTTGCAT TCCCCTCTTC CCAATTCCCA TAGAGAAGAT
1401  TATTTATCCT AACGATTGGT GGACGCTAAG TCCCTGCTGT TTTGATTATA
1451  TATCAAATGT TGAAACAAAT TTTGTTTAGT TTCTTTTTGT ACTCTAAAAA
1501  GAAGACAAAA AATTCTTTAT AAACTGTACA CTCTAAACAA AATAGTTCAC
1551  AATAAACAGC AATACATTAT AATTAATTGG AGGATACTAT TGTCATGAAC
1601  CTGAAACCTT TGAATGACCG TGTTTTAGTA AAACGTCTTG AATCTGAAGA
1651  AAAAACAGCT GGTGGACTCT ATATCCCTGA TACTGCTAAA GAAAAACCAT
1701  CTCGTGGTGA AGTTGTTGCT GTTGGACCTG GTAAACATAC AGATGATGGT
1751  AAATTAATAC CTATGGCTGT AAAAGCAGGA GATACAGTTC TTTTTAATAA
1801  GTATGCAGGA ACAGAAGTAA AGCTTGATGG TGTAGAGCAT CTAGTTATGC
1851  GTGAAGATGA CATCCTAGCT GTTATTACTG GAGAAACTGG CCGCAAGTGA
1901  AAAAGGCGTA AATAAAAAGA TCGGTGATCT TTAATAATTT TATTCAGTTA
1951  TAATGAAAAC ACTAATTACA CGCACTCTCT GAGAATTTTC TCAGAAAACT
2001  ATATTTAACA ATTCTAAAAT CGATATGTTT TTAGGAGGAA AACCCTAATG
2051  GCTTCTAAAG AAATCCTTTT TGATGCTAAA GCCCGTGAAA AACTTTCACG
2101  AGGTGTAGAT AAACTTGCAA ATGCTGTTAA AGTAACACTT GGACCTAAAG
2151  GCCGTAATGT CGTTATTGAA AAGTCTTTTG GTTCCCCAGT TATTACAAAA
2201  GATGGTGTAT CTGTTGCAAA AGAAATTGAA CTTGAAGATA AGTTTGAAAA
2251  TATGGGCGCT CAAATGGTTA AAGAAGTAGC TCCCAAAACT AGCGATATTG
2301  CTGGTGATGG AACTACAACA GCAACAGTCC TTGCACAAGC TATTTATCGT
2351  GAAGGTGTAA AACTTGTAGC AGCTGGTCGT AATCCTATGG CCATTAAACG
2401  TGGCATAGAT AAAGCTGTTG TTGCTGTTAC TAAAGAACTA AGCGACATTA
2451  CAAAGCCTAC TCGTGACCAA AAAGAAATAG CTCAAGTTGG AACCATTTCT
2501  GCAAACTCTG ATACAACAAT AGGTAATATC ATAGCTGAAG CTATGGCTAA
2551  AGTTGGAAAA GGAGGTGTTA TCACAGTTGA GGAAGCTAAA GGTCTTGAAA
2601  CTACATTAGA TGTGGTTGAA GGAATGAAGT TTGACCGTGG CTACCTCTCT
2651  CCATACTTTG TAACTAATCC TGAGAAAATG GTTTGTGAAC TTGATAACCC
2701  TTATATCCTT TGTAATGAGA AAAAGATTAC TAGCATGAAA GACATGCTAC
2751  CAATCTTAGA ACAAGTTGCT AAAGTAAACC GTCCACTCCT TATTATTGCT
2801  GAAGACGTAG AAGGTGAAGC ACTTGCAACA CTTGTAGTCA ATAAGCTCCG
2851  TGGAGCACTC CAAGTTGTAG CCGTAAAAGC TCCTGGTTTT GGTGAACGCC
2901  GTAAAGCTAT GCTTGAAGAT ATTGCTATCC TTACTGGAGG AGAAGCAATA
2951  TTTGAAGATC GTGGTATAAA GCTTGAAAAT GTAAGCTTGT CTTCTTTAGG
3001  AACAGCTAAA CGTGTAGTTA TTGACAAAGA AAATACTACT ATCGTTGATG
3051  GTGCTGGAAA ATCAGAAGAT ATTAAAGCTC GAGTTAAACA AATTCGTGCA
3101  CAAATTGAAG AAACAAGCTC AGATTATGAT CGTGAAAAAC TTCAAGAACG
3151  TCTTGCAAAA CTTGTTGGTG GAGTAGCTGT TATCCATGTT GGAGCTGCTA
3201  CTGAAACTGA AATGAAAGAG AAGAAGGATC GTGTAGAAGA TGCTCTAAAT
3251  GCAACAAGAG CTGCGGTTGA AGAAGGTATT GTCCCTGGTG GTGGTACTGC
3301  TTTTGTCCGC TCCATTAAAG TCCTTGATGA TATTAAACCT GCTGATGATG
3351  ATGAACTTGC TGGACTTAAT ATCATCCGTC GTTCTCTTGA AGAGCCTTTA
3401  CGTCAAATTG CTGCAAATGC TGGCTATGAA GGTTCTATTG TTGTAGAAAA
3451  AGTTCGTGAA CCAAAAGATG GTTTTGGATT TAATGCTGCA TCAGGAGAAT
3501  ATGAAGACCT TATTAAAGCT GGTGTCATTG ATCCTAAAAA AGTTACACGT
3551  ATTGCATTAC AAAATGCAGC ATCAGTAGCC TCCTTACTTC TAACTACAGA
3601  ATGCGCTATT GCTGAAAAAC CAGAACCTAA AAAAGATATG CCTATGCCTG
3651  GCGGTGGTAT GGGTGGTATG GGTGGTATGG ACGGTATGTA CTAGTCCTAT
3701  CTTCAGTACA ACTTAGATGT ATAAAAACCC CAGAAGCAAT GCTTCCGGGG
3751  TTTTATACTT TCAGCATAAA AAATTAATAT TTAATATACA GACACATTAT
3801  TTTGGTATTT ATTATTTATT ATGATCAAAT ATATAGACTG GATACAAAAA
3851  ACAACAATGA TGTTTAAAAA GGCAGGGATA GATTCACCAA AACTCTCTGC
3901  AGAACTTATA TTAAGTCATG TTTTAAATAT TACACGATTA CAAATAATAA
3951  TGACTCCTTT TGAACCTATT CCAACTAATA GCTACTCAAC GCTTAATGAT
```

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4001  ATCATGTTAA GAAGACTCCA TGGAGAACCA ATTGCATATC TCACAGGGAA
4051  AAAAGAATTT TTTTCACGAG AATTTAAAGT CACTCAAGCC ACACTTATCC
4101  CTCGCCCAGA GACAGAGTTA CTTATAGAAT TTGTATTAAA CCATATTAAC
4151  CCAACACAAC AAATATACTT TGCAGACTTA GGTACAGGTA GTGGGTGTAT
4201  TGCAATTTCA CTAGCTGCTG AAAGAAAAAA TTGGTTAGGT ATTGCTACTG
4251  ATATCTCTAG TGAAGCATTA AAAATAGCTA AACTTAATAG TTTAAAAAAT
4301  AACACTCATA GTCAACTACA GTTTCTTCAA TCAGATTTTA CACAACCACT
4351  CTGTCTACCC TCTTCATTAG ACTTATATAT CAGTAATCCT CCATATATAA
4401  GTGAAAATGA ACTGACCTCT CTTCCGCATG AAGTAATATC TTTTGAACCT
4451  AAAATAGCTC TTACACCACA TAAATGTATT CATCTTGATG AAATAAATAC
4501  CGTTTTACAC TGCTATAAAA AAATTATTAC CCAAGCAGAG ATATCCCTTA
4551  AGCCTGGAGG AATAATAATT TTAGAACATG GAGCAACACA AGCAGAAGCT
4601  ATCTTATTGT TGTTAAAAAA CAACATATGG ACAAATGTAA TAAGTCATAC
4651  TGATCTTACA AATAAAAATC GTTTTATTAC AGCATATAAG TATAAAATAT
4701  AACTTAATTA TGTTGkagAa AAAACAAAAA ATAAAAATAA GATATTAAaT
4751  ATTTtttttA aTAAAATTAA GCAAtTACTA ATATCTTTTT TTGGrTCGtt
4801  yaTtGsATwA GAAACTTTGG rGGrTrrCTa TGAACAAACA ACCATnCAAC
4851  GGCCAAnTAC ATnnCAGGnT TGGGGTCATA GGGGCCACGC TTTATGTACG
4901  TACAACCCCn ACTGAAATTC TGGnTTGnTT TGGGGGGnAA nTGGGTATCG
4951  CAACnCTnTC CCCCCCCCCT GG
```

DATED this 30th day of November, 1995

DARATECH PTY. LTD. and Pig Research and Development Corporation

By Its Patent Attorneys

DAVIES COLLISON CAVE

AUSTRALIAN PATENT OFFICE SEC. 104

FIGURE 1

```
ATG GCT TCT AAA GAA ATC CTT TTT GAT GCT AAA GCC CGT GAA AAA CTT        48
Met Ala Ser Lys Glu Ile Leu Phe Asp Ala Lys Ala Arg Glu Lys Leu
  1               5                  10                  15

TCA CGA GGT GTA GAT AAA CTT GCA AAT GCT GTT AAA GTA ACA CTT GGA        96
Ser Arg Gly Val Asp Lys Leu Ala Asn Ala Val Lys Val Thr Leu Gly
             20                  25                  30

CCT AAA GGC CGT AAT GTC GTT ATT GAA AAG TCT TTT GGT TCC CCA GTT       144
Pro Lys Gly Arg Asn Val Val Ile Glu Lys Ser Phe Gly Ser Pro Val
         35                  40                  45

ATT ACA AAA GAT GGT GTA TCT GTT GCA AAA GAA ATT GAA CTT GAA GAT       192
Ile Thr Lys Asp Gly Val Ser Val Ala Lys Glu Ile Glu Leu Glu Asp
     50                  55                  60

AAG TTT GAA AAT ATG GGC GCT CAA ATG GTT AAA GAA GTA GCT CCC AAA       240
Lys Phe Glu Asn Met Gly Ala Gln Met Val Lys Glu Val Ala Pro Lys
 65                  70                  75                  80

ACT AGC GAT ATT GCT GGT GAT GGA ACT ACA ACA GCA ACA GTC CTT GCA       288
Thr Ser Asp Ile Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala
                 85                  90                  95

CAA GCT ATT TAT CGT GAA GGT GTA AAA CTT GTA GCA GCT GGT CGT AAT       336
Gln Ala Ile Tyr Arg Glu Gly Val Lys Leu Val Ala Ala Gly Arg Asn
            100                 105                 110

CCT ATG GCC ATT AAA CGT GGC ATA GAT AAA GCT GTT GTT GCT GTT ACT       384
Pro Met Ala Ile Lys Arg Gly Ile Asp Lys Ala Val Val Ala Val Thr
        115                 120                 125

AAA GAA CTA AGC GAC ATT ACA AAG CCT ACT CGT GAC CAA AAA GAA ATA       432
Lys Glu Leu Ser Asp Ile Thr Lys Pro Thr Arg Asp Gln Lys Glu Ile
    130                 135                 140

GCT CAA GTT GGA ACC ATT TCT GCA AAC TCT GAT ACA ACA ATA GGT AAT       480
Ala Gln Val Gly Thr Ile Ser Ala Asn Ser Asp Thr Thr Ile Gly Asn
145                 150                 155                 160

ATC ATA GCT GAA GCT ATG GCT AAA GTT GGA AAA GGA GGT GTT ATC ACA       528
Ile Ile Ala Glu Ala Met Ala Lys Val Gly Lys Gly Gly Val Ile Thr
                165                 170                 175

GTT GAG GAA GCT AAA GGT CTT GAA ACT ACA TTA GAT GTG GTT GAA GGA       576
Val Glu Glu Ala Lys Gly Leu Glu Thr Thr Leu Asp Val Val Glu Gly
            180                 185                 190

ATG AAG TTT GAC CGT GGC TAC CTC TCT CCA TAC TTT GTA ACT AAT CCT       624
Met Lys Phe Asp Arg Gly Tyr Leu Ser Pro Tyr Phe Val Thr Asn Pro
        195                 200                 205

GAG AAA ATG GTT TGT GAA CTT GAT AAC CCT TAT ATC CTT TGT AAT GAG       672
Glu Lys Met Val Cys Glu Leu Asp Asn Pro Tyr Ile Leu Cys Asn Glu
    210                 215                 220

AAA AAG ATT ACT AGC ATG AAA GAC ATG CTA CCA ATC TTA GAA CAA GTT       720
Lys Lys Ile Thr Ser Met Lys Asp Met Leu Pro Ile Leu Glu Gln Val
225                 230                 235                 240

GCT AAA GTA AAC CGT CCA CTC CTT ATT ATT GCT GAA GAC                   759
Ala Lys Val Asn Arg Pro Leu Leu Ile Ile Ala Glu Asp
                245                 250
```

FIGURE 2 ISI GroES

```
  M    N    L    K    P    L    N    D    R    V    L
ATGAACCTGAAACCTTTGAATGACCGTGTTTTA

  V    K    R    L    E    S    E    E    K    T    A
GTAAAACGTCTTGAATCTGAAGAAAAAACAGCT

  G    G    L    Y    I    P    D    T    A    K    E
GGTGGACTCTATATCCCTGATACTGCTAAAGAA

  K    P    S    R    G    E    V    V    A    V    G
AAACCATCTCGTGGTGAAGTTGTTGCTGTTGGA

  P    G    K    H    T    D    D    G    K    L    I
CCTGGTAAACATACAGATGATGGTAAATTAATA

  P    M    A    V    K    A    G    D    T    V    L
CCTATGGCTGTAAAAGCAGGAGATACAGTTCTT

  F    N    K    Y    A    G    T    E    V    K    L
TTTAATAAGTATGCAGGAACAGAAGTAAAGCTT

  D    G    V    E    H    L    V    M    R    E    D
GATGGTGTAGAGCATCTAGTTATGCGTGAAGAT

  D    I    L    A    V    I    T    G    E    T    G
GACATCCTAGCTGTTATTACTGGAGAAACTGGC

  R    K    *
CGCAAGTGA
```

FIGURE 3

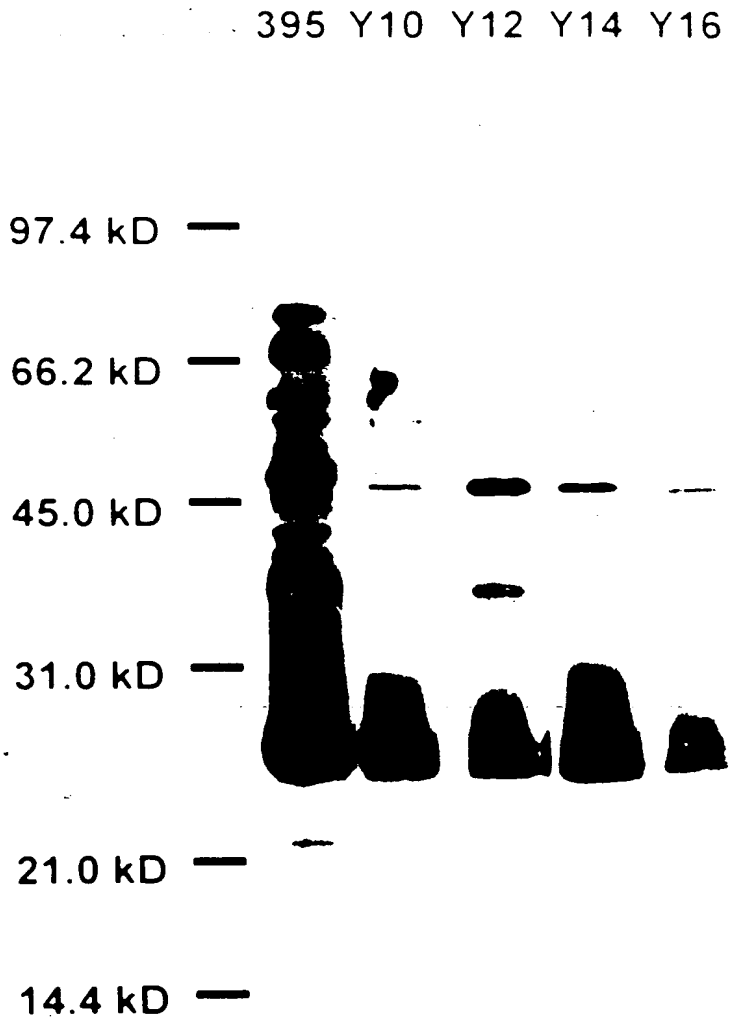


FIGURE 4

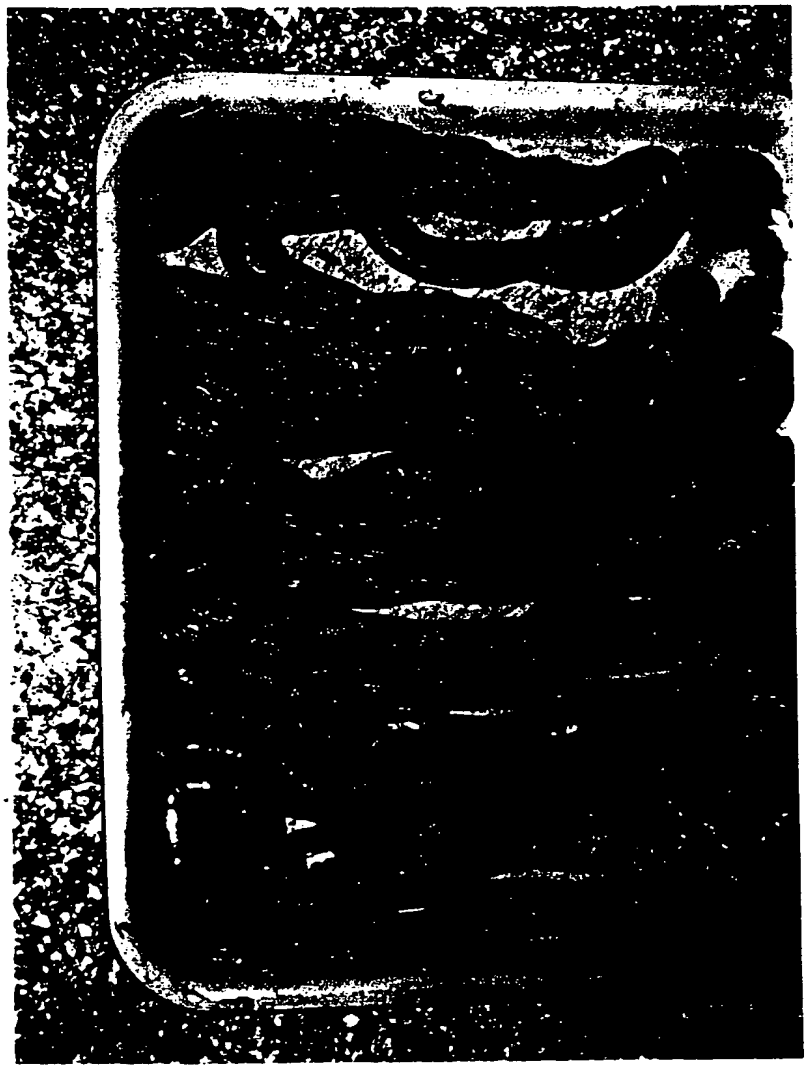


FIGURE 5

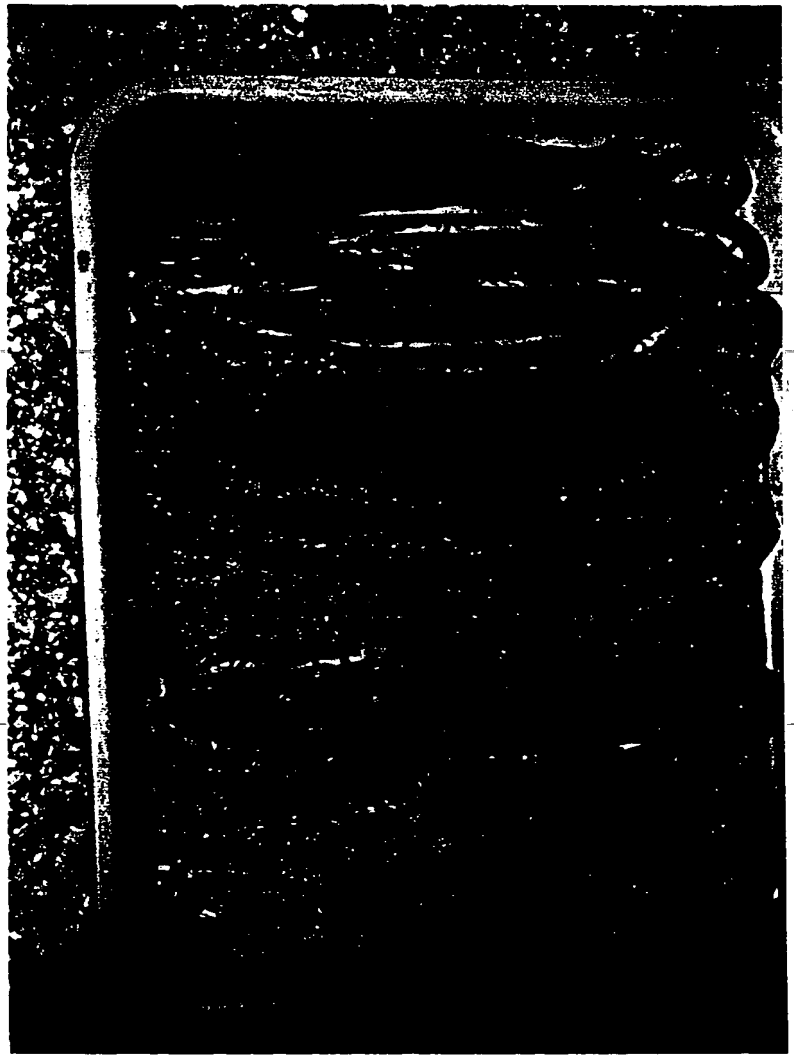
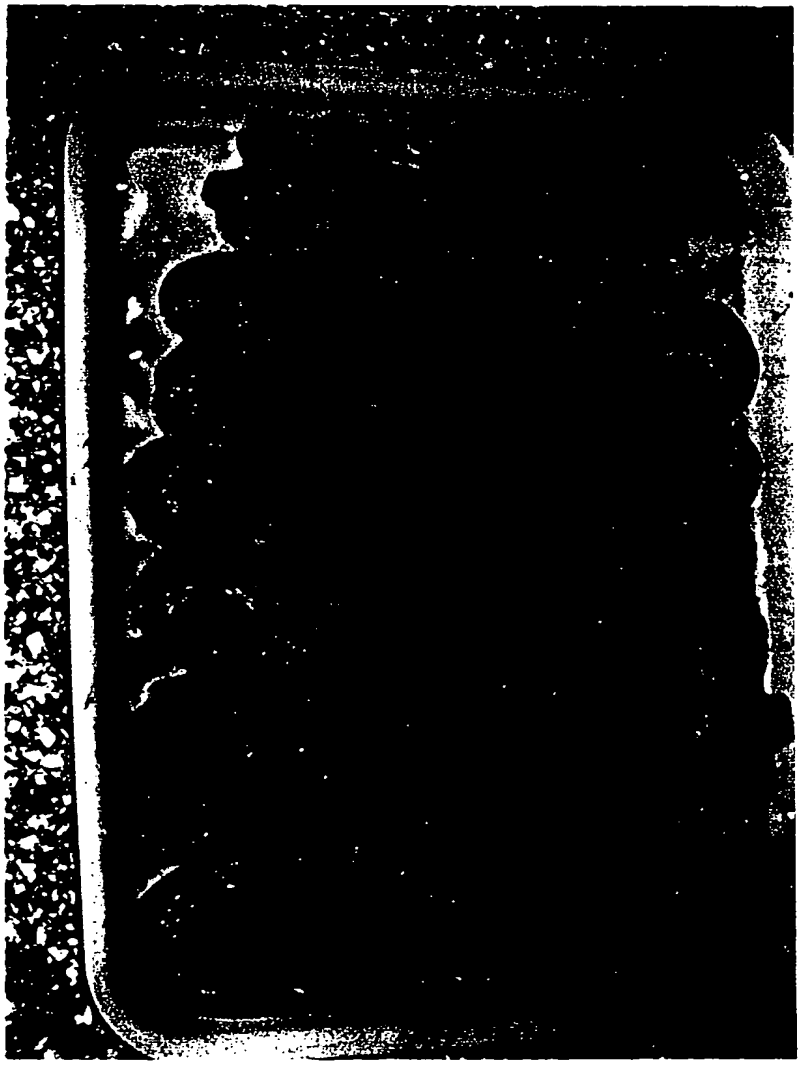
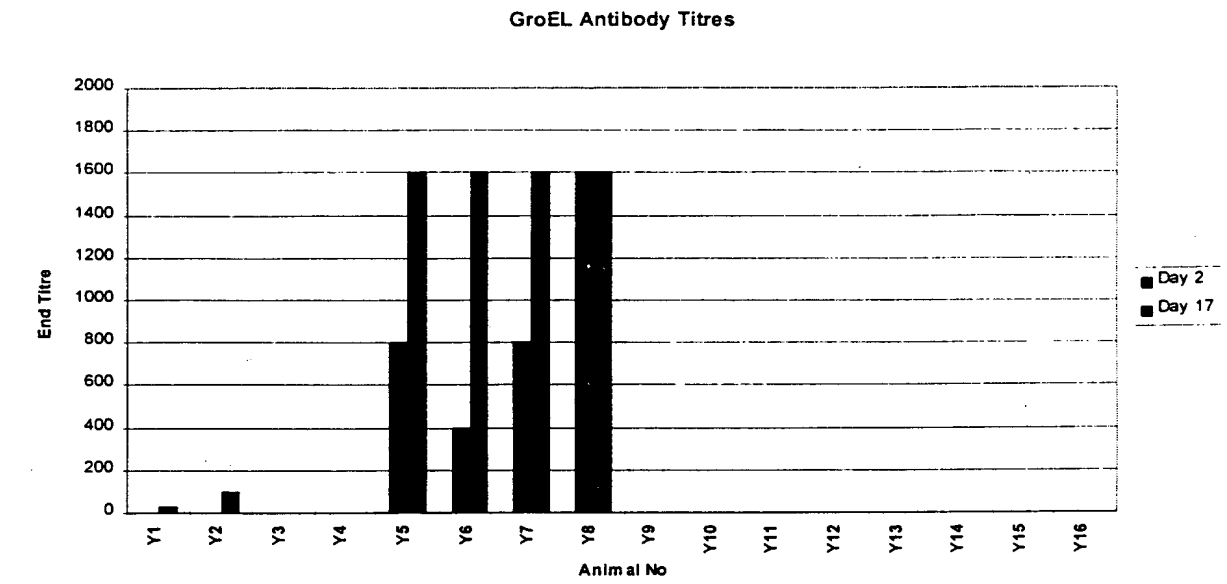


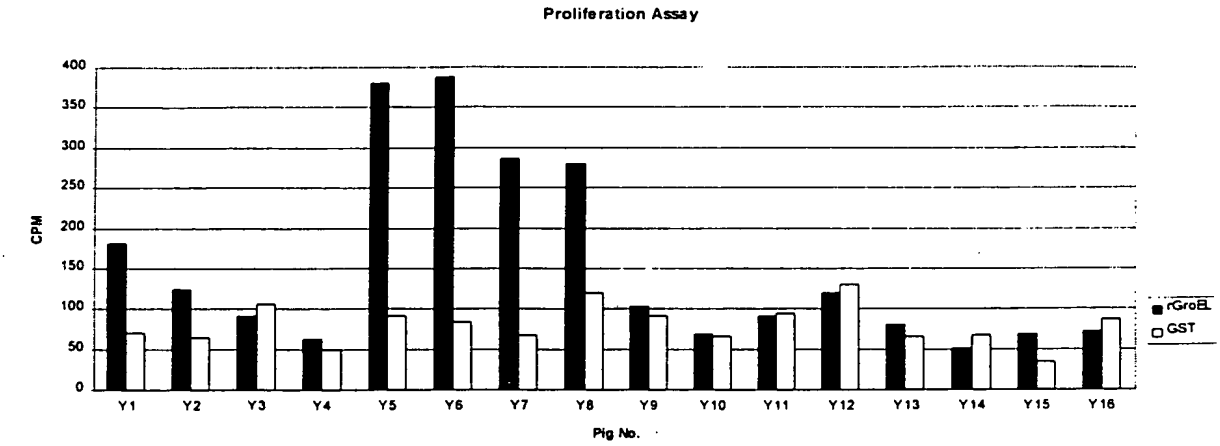
FIGURE 6





End titres of GroEL antibodies at day 2 and day 17

FIGURE 7



T-cell proliferation responses to GroEL and GST proteins by peripheral blood lymphocytes obtained from pigs on day 2.

FIGURE 8

Figure 9

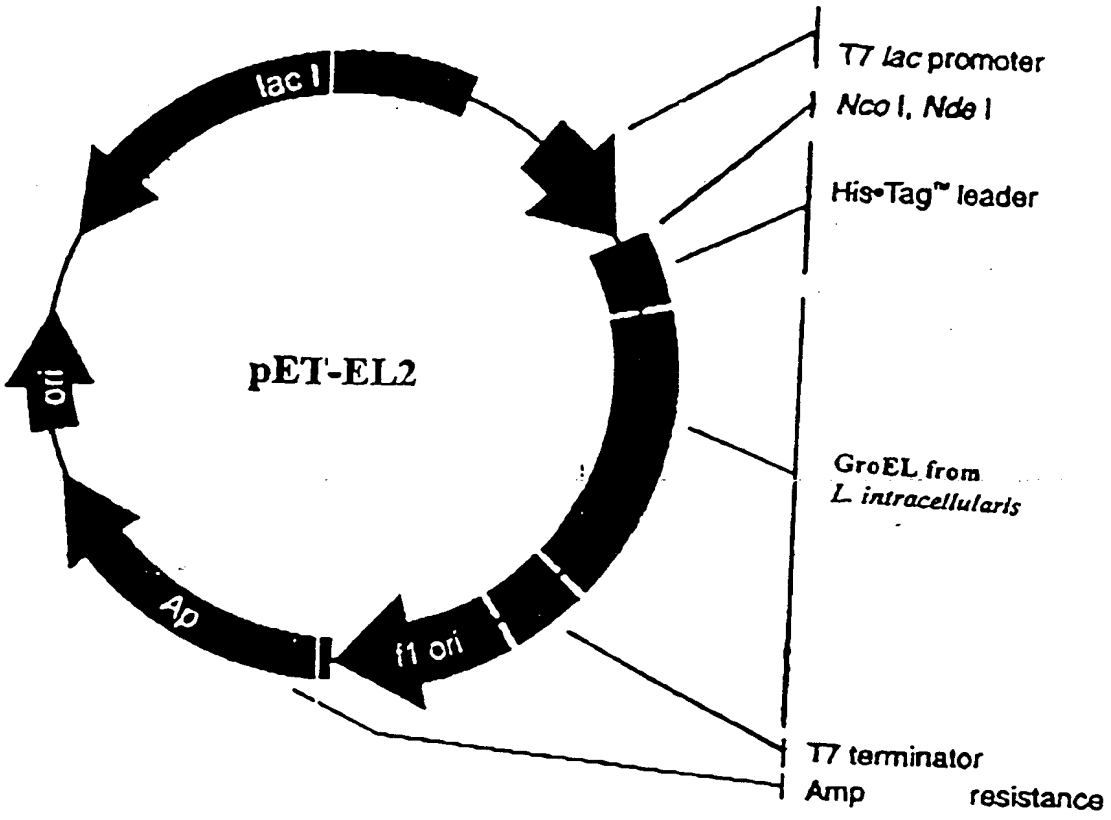

lac I
ori
pET-EL2
Ap
f1 ori
T7 lac promoter
Nco I, Nde I
His•Tag™ leader
GroEL from
L. intracellularis
T7 terminator
Amp resistance

Figure 10